Transformations of the texture and the mineralization of the dentary bone in the Atlantic salmon, *Salmo salar* L. (Salmonidae), during anadromous migration

by

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ABSTRACT. - During its anadromous migration, the Atlantic salmon, *Salmo salar*, shows morphological transformations that affect various parts of its skeleton. The most spectacular concerns the jaws that lengthen and thicken to form a "kype" in the males. During this time, scales and vertebrae show important erosion in the males as in the females. The aim of the present work was to verify if the dentary has a positive osseous global assessment: what is the true balance between osteogenesis and resorption during the anadromous migration? An analysis of the dentary mineralising rate (DMR), the dentary bone compactness (DBC) and the mineralization degree (MDD) of dentary bone on fish caught in the Scorff River (Brittany, France) at the beginning of their ascent and after spawning, shows two main phenomenons: *a*) a conspicuous increase of the bone compactness; this means that the quantity of osseous substance during the anadromous migration, in males as in females, is higher. This is probably the result of an increase of osteogenesis, but it also could be a decrease of bony destruction by osteoclastic cells or, even a grouping of both processes; *b*) a small loss of mineral: the TDM decreases. This loss can have two origins: *i*) an incomplete mineralization of new bone (the maturation of hydroxyapatite crystals is not achieved); *ii*) a diffuse demineralization (or halastasy) of old bone. An osteoclastic activity releases both organic components and mineral ions that give a null balance for the TDM, whereas the halastasy attacks only the mineral component. In fact these two hypotheses (unmatured new bone and halastasic old bone) can act in synergy. Lastly, the evolution of the histological characteristics of dentary bone are practically the same in the two sexes except for the mineralization degree that is slightly higher in the females than in males.

RÉSUMÉ. - Étude des transformations de la texture et de la minéralisation de l'os des dentaires chez le saumon atlantique, *Salmo salar* L. (Salmonidae), pendant la migration anadrome.

Pendant sa migration anadrome, le saumon atlantique, *Salmo salar*, présente des transformations morphologiques qui affectent diverses parties de son squelette. Les plus spectaculaires concernent les mâchoires qui s'allongent et s'épaississent, pour former un "bec" chez les mâles. Pendant cette période, les écailles et les vertèbres sont soumises à une importante érosion, chez les mâles comme chez les femelles. Le but du présent travail était de vérifier si le dentaire du saumon atlantique a un bilan osseux positif : quel est le véritable équilibre entre ostéogenèse et résorption sur cet élément squelettique, durant l'étape de migration anadrome ? Une analyse du taux de minéral (TDM), de la compacité osseuse (CO) et du degré de minéralisation (DDM) du dentaire d'individus capturés dans le Scorff (rivière côtière de Bretagne, France) au début de leur migration anadrome et après la reproduction, montre deux phénomènes principaux : *a*) une très nette augmentation de la compacité osseuse, ce qui signifie que la quantité de substance osseuse augmente, pour les deux sexes, pendant la remontée en rivière jusque sur les lieux de la fraie. C'est probablement le résultat d'une augmentation de l'ostéogenèse, mais cela peut aussi résulter d'une baisse significative des processus de résorption osseuse par les ostéoclastes, sans négliger la possibilité d'une synergie de ces deux facteurs ; *b*) une légère perte de minéral : le TDM décroît. Cette perte peut avoir deux origines : *i*) une minéralisation incomplète de l'os nouveau (la maturation des cristaux d'hydroxyapatite n'est pas achevée) ; *ii*) une déminéralisation diffuse (ou halastasie) de l'os ancien. Effectivement, l'activité ostéoclasique libère à la fois des composants organiques et des ions minéraux, ce qui donne un bilan nul pour le TDM, tandis qu'une activité halastasique ne fournit que le composant minéral sans dégradation du support organique de l'os. En fait, ces deux hypothèses (de l'os nouveau immature et d'une action halastasique sur l'os ancien) peuvent agir en synergie. L'évolution des caractéristiques histologiques de l'os du dentaire est pratiquement identique dans les deux sexes sauf pour le degré de minéralisation qui est légèrement plus élevé chez les femelles que chez les mâles.

Key words. - Salmonidae - *Salmo salar* - France - Scorff River - Dentary - Mineralization - Migration - Kype.

A number of morphological transformations affecting skeletal elements are linked to genital maturation during anadromous migration of the Atlantic salmon, *Salmo salar* (Tchernavin, 1938b, 1944; Fleming, 1996). The most spectacular of these transformations affect jaws that become a "bill", the "kype", in male salmons. The lengthening of the anterior regions of upper and lower jaws is the outcome of an hyperplasy of *i*) the ethmoidian cartilages, *ii*) the dentary

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and premaxillary bones and *iii*) the mandibular symphysis (Tchernavin, 1944; Fontaine, 1948; Fleming, 1996). Tchernavin (1938a) also noticed that some of the bones in the skull are reducing at the same time of the formation of the kype: bones of the gill-covers, branchiostegals and postorbitals.

Bone hyperplasy would involve an increase in osteogenesis: deposition of a collagen matrix and its mineralization (Witten and Hall, 2002; 2003). Organisms should therefore mobilize various components to construct new bone: organic matrix (collagen and proteoglycans) and mineral elements for hydroxyapatite crystals. However, neither the organic components nor the mineral components have an alimentary origin because salmons do not eat during spawning anadromous migration (Fontaine, 1948; Fleming, 1996). These mineral ions would be taken a) from external surroundings (at gill epithelium and tegument) but the freshwater is very poor in dissolved minerals; b) and/or directly from the organism storage (skeleton *sensu lato*). It has been shown that scales demineralize during this phase of the fish's life cycle (Crichton, 1935; Van Someren, 1937; Persson *et al*., 1999; Kacem, 2000); the scales would provide a part of ions, but also of organic components, necessary to carry out the morphological transformations quoted above. Furthermore, it was recently shown that vertebral skeleton also contributes to the turn-over of organic and mineral components of bone tissue, as it undergoes significant phenomenons of osteoclastic resorption (Kacem *et al*., 1998), osteocyte osteolysis (Kacem and Meunier, 2000) and halastasic demineralization (Kacem *et al*., 2000; Kacem and Meunier, 2003). Both males and females are affected by this destruction of mineralized tissues (Kacem *et al*., 1998). While in males it would be involved in the formation of the kype, in females the mobilized calcium would be involved in vitellogenesis (Persson, 1997).

Another characteristic phenomenon is the turnover that affects teeth during the migration. Tchernavin has described a toothless stage at the beginning of the anadromous ascent of the salmon and a second period with the formation of "breeding teeth" (Tchernavin, 1938b). But this intermediate toothless stage is contested by Witten *et al*. (2005) which considered that the "complete tooth loss / replacement" observation results, in first hand, from the proliferation of the oral mucosa and so conceals the teeth prior the breeding period, and, in second hand, to an artefactual consequence of maceration techniques that could have removed all teeth with an incompletely mineralised base.

The aim of the present paper is to test whether the dentary shows only an increase of bone material during the anadromous migration or if it also shows simultaneous resorption-reconstruction somewhere. This study was managed in males and females to analyse an eventual sexual difference in the bone biology of the dentary. With this end in view, we have analysed the evolution of bone compactness and of mineral content of the dentary bone of male and female salmons from the Scorff River (Brittany) at the beginning of the anadromous migration and compared them to a sample of specimens coming from the breeding area. Moreover, to evaluate the possible implication of the anadromous migration process in the histological changes of the jaws, we also have studied seawater reared salmons that have undergo breeding directly after immersing in freshwater.

MATERIALS AND METHODS

Material

Salmons used in this study were collected in the Scorff River (Brittany, France) (Tab. I). It is a small river, 75 km long, with a catchment area of 480 km^2. Its estuary is about 15 km long. A salmon trap was set up at "Moulin des Princes" Pont-Scorff (03°24'07" W; 47°50'06" N). The area of colonisation and reproduction of Atlantic salmons (*Salmo salar*) extended to 50 km up the river of the salmon trap.

From 1994 to 1998, twenty-nine salmons (8 males and 21 females) were collected at the beginning of their spring anadromous migration. These samples were realised in the period stretched between April and July. We will call these specimens "Ascending Salmon" oppositely to the thirty-three "Spawning Salmon" (18 females, 14 males) that were collected at the end of their anadromous migration at the breeding areas, usually after their gametes emission, between August and December.

Salmons were weighed, sexed and precisely measured to the nearest mm (the fork length ranged from 505 to 955 mm). To estimate the individual age and the number of years these fishes lived in the sea, some scales were taken from salmon's sides, below the anterior limit of the dorsal fin and above the lateral line (Baglinière, 1985; Ombredane and Baglinière, 1992). Among the 61 specimens, 37 were grilses (salmons that have spent one winter at sea before returning to spawn for the first time) and 24 were spring salmons (animals that were at least two years in the sea).

In order to complete our sample and to test the impact of the anadromous migration factor, which needs a high energetic expense, Favot Salmon Culture Centre (Finistère, France) provided us with thirty-five reared salmons. After

Table I. - The sample of Atlantic salmon caught in the Scorff River (Brittany). *[Répartition des saumons sauvages échantillonnés à la rivière du Scorff (Bretagne).]*

Stage of migration	Beginning		End		Total
Age	Grilse	Spring salmon	Grilse	Spring salmon	
Males	4	4	11	3	22
Females	10	11	12	6	39
Total	29		32		61

smoltification in freshwater (one year), these animals were reared one year and a half in the sea and transferred to fresh water where they spawn. These salmons did not undergo hormonal treatment neither during the growth phase nor during the reproductive phase. Some of these immature salmons were collected in April 1996, during the phase of growth in the sea (8 females and 10 males). The others (9 females and 8 males) were collected in freshwater after the reproductive phase in January 1997.

Methods

Dentary mineralization rate (DMR)

All the dentary bones are dehydrated in a graded series of alcohol; fat was removed through several washings in acetone and trichloroethylene and, after, the bones were dried. For each dentary the number of functional teeth (fused to the bone) is recorded. The count of these teeth is a very important data for our results interpretation (see below). The left dentaries were weighed to within 10^{-2} mg (dry weight), then incinerated for 6 h in a muffle furnace (750°C). The ashes obtained were weighed to within 10^{-2} mg (mineral weight). The dentary mineral rate (DMR%) is calculated as follows:

DMR% = Mineral weight / Dentary dry weight x 100

In order to compare the total weight of the dentary and its mineral part according to the length of each specimen, two ratios have been calculated, RD1 and RD2:

$$RD1 = DW / FL^3$$
$$RD2 = MDW / FL^3$$

(DW = Dentary dry weight, g; FL = Fork Length, mm; MDW = Mineral dentary weight, g)

Dentary bone compactness (DBC) and histology

Like other Salmonidae, salmon dentary is a roughly triangle-shaped bone with a relatively thick anterior region whereas the posterior one gets thinner and ended by two spindled processes (ventral and dorsal). These processes lean respectively on the dorsal and the ventral side (Francillon, 1977). Four characteristic and comparable sections levels were determined for each specimen to ascertain the dentary texture and the different types of hard tissues that constitute the skeleton of the kype. They are situated between the symphysal anterior area and the base of the two posterior processes of the dentary (Fig. 1).

The right dentaries were impregnated successively in styrene, three successive baths of increasing concentration (25%, 50%, 75%) of Stratyl 'chronolite 2195', then in pure stratyl. Bones were embedded in a polyester resin (98% stratyl, 2% Luperox catalysor), and sectioned in the four levels with a Leitz 1600-saw microtome into slices of 125 ± 10 μm. Then these slices were microradiographed with CGR Sigma 2060 generator. The mineralized areas (bony tissue) appear

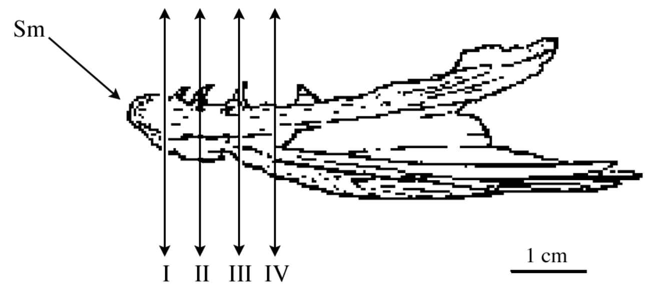


Figure 1. - Drawing of a dentary and location of the four levels of sectioning. *[Dessin du dentaire et localisation des quatre niveaux de coupe.]*

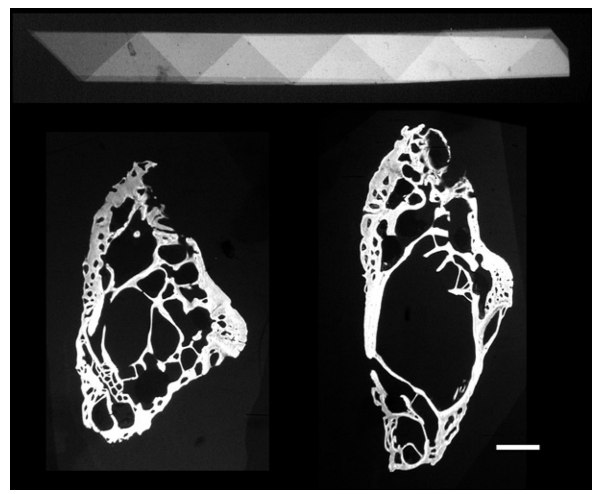


Figure 2. - Microradiograph of two dentary sections with the aluminium standard. (Scale bar = 1 mm). *[Microradiographie de deux coupes de dentaire avec l'étalon d'aluminium standard. (Échelle = 1 mm).]*

in various grey density in the microradiographs according to their mineral density, whereas the unmineralized areas appeared black (Fig. 2). Images of these microradiographs were digitalized using an Olympus Camedia digital camera mounted on an Olympus SZX12 binocular microscope. A software package for image analysis (NIH Image 1.63) was used to quantify the dental compactness by measuring the surface of bone tissue (grey areas) according to the total surface of the bone section without the teeth surface. Then the compactness of each dentary section is calculated:

% DBC= SBT / TSS x 100

(DBC = Dentary Bone Compacity; SBT = Surface of bone tissue; TSS = total surface of the section)

Differences in mineral rate between male and female salmon at the beginning and at the end of the anadromous migration were calculated using one way ANOVA for each variable: sex, migration stage and age (Statview version 4.02). The percentage values of mineral rate and the dentary bone compactness were transformed using arcsinus of the square root to normalise the value (Sokal and Rohlf, 1995).

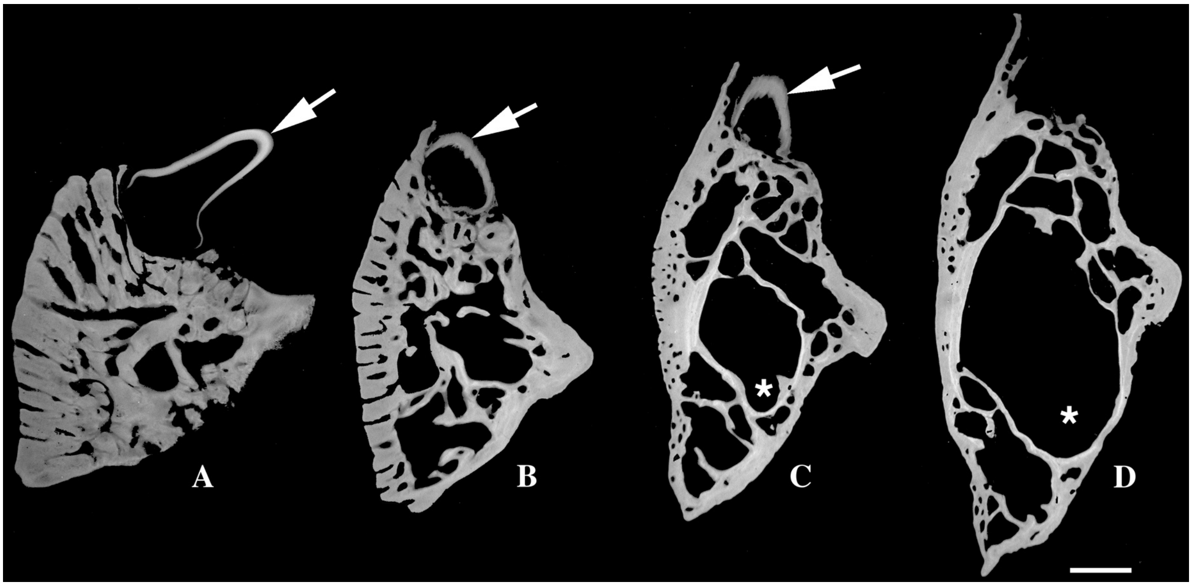


Figure 3. - Microradiography of the four sections of a dentary A, B, C and D, levels I, II, III and IV, respectively. Medulla is practically lacking on level I. Three functional teeth are shown on the three first sections (arrows). Location of Meckel's cartilage is pointed by an asterisk. (Scale bar = 1 mm). *[Microradiographie des quatre sections d'un dentaire : A, B, C et D niveaux respectifs I, II, III et IV. La zone médullaire est pratiquement absente sur le niveau I. Trois dents fonctionnelles sont visibles sur les 3 premières coupes (flèches). La localisation du cartilage de Meckel est pointée par un astérisque. (Échelle = 1 mm).]*

Mineralization degree of dentary (MDD)

To determine the mineralization degree of bone, we used quantitative microradiography (Rasmussen, 1970; Boivin and Baud, 1984; Kacem and Meunier, 2003). The technique consisted of comparing the degree of blackening of microradiographs to that of an aluminium standard of increasing thickness (Fig. 2). The precedent sections of 125 μm (see before) were ground manually till 100 ± 1 μm thick; quantitative microradiography requires thin parallel faced sections (Jowsey *et al.*, 1965; Boivin and Baud, 1984). When this accurate thickness was reached, the sections were cleaned in 70% alcohol to remove the fine abrasive powder grains, and eventually the glass dusty fragments; they were held between two glass slides. The sections were microradiographied with a CGR Sigma 2060 generator. The generated rays were copper Kα-rays filtered through a nickel sheet to obtain a monochromatic wavelength of 1.54 Ås^{-1} (Rasmussen, 1970; Boivin and Baud, 1984). The thin sections of bone and the aluminium standard were brought into contact with a fine-grain photographic emulsion (film with high resolution Kodak HRP SO 343), that was place on a holding plate. The whole set was wrapped in a X-rays transparent fine sheet. Then a vacuum was created in the set so that the film, the section and the aluminium standard kept their initial position in spite of rotation of the plate. Holding plates were then put, three by three, on a turning plate for a slow and continuous rotation under vacuum. X-rays were therefore spread uniformly on the three holding plates. Under these conditions, the exposure time was 60 min, the distance from the X-ray source was 40 cm, the intensity was 7 mA and the tension was 20 kV. After exposure, the films were developed for 5 min at 20°C. Then, microradiographs were fixed, rinsed and dried. Finally, each microradiograph was put between a slide and cover glass for analysis. To measure the degree of mineralization, the microradiographied bone areas were compared to the optical density of different thickness of aluminium standard (50, 62.5, 75, 87.5, 100, 112.5, 125, 137.5 and 150 μm) (Bohatirchuk, 1966; Boivin and Baud, 1984; Meunier and Boivin, 1997) (Fig. 2). The aluminium standard employed was made from 12.5 μm thick 99.9% pure foil. A triangle of this foil was folded several times in order to obtain the standard with successive triangular steps of increasing thickness. Each thickness had a different X-ray absorption. The ratio of the mass absorption coefficient of pure aluminium to that of bone hydroxyapatite was fairly constant (c = 0.561) with monochromatic X-ray radiation (copper Kα-ray = 1.54 Å, Nickel filter) (Sissons *et al.*, 1960; Rasmussen, 1970; Boivin and Baud, 1984). Thus, the degree of mineralization, i.e. the quantity of mineral substance present in a unit of bone volume (g mineral cm^{-3} bone) could be determined (Sissons *et al.*, 1960). On each microradiograph, areas 100 ±1 μm thick, outside of the eroded osteoclastic surfaces (Howship's lacunae) and outside of the main osteolytic lacunae (significant hypomineralized patches), were digitalized by an Olympus Camedia digital camera mounted on an Olympus SZX12 binocular microscope and analysed by NIH-Image 1.63.

RESULTS

General structure

As described by the precedent authors, the main change in the morphology of dentary concerns the anterior region of the bone that swallows to form the ventral part of the kype, the dorsal part of which results essentially from the premaxillary bone. The posterior part of the dentary keeps unchanged.

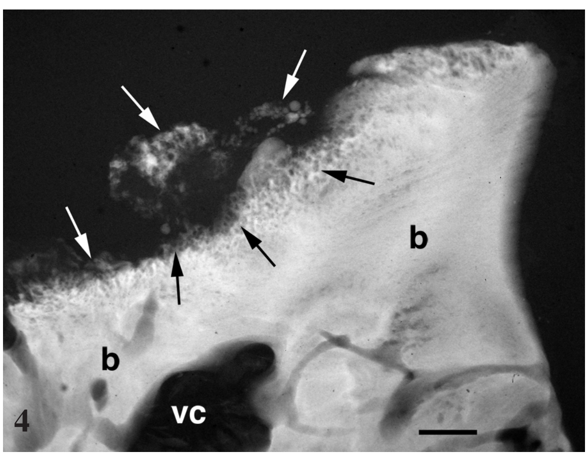
Teeth number on dentaries is highly variable according to the migration stage. Thus, as already mentioned by Tchernavin (1938b), the wild ascending salmons have few or nearly no teeth (an average of one tooth for both males and females) whereas the spawning salmons present generally a full set of attached teeth (9 to 10 teeth).

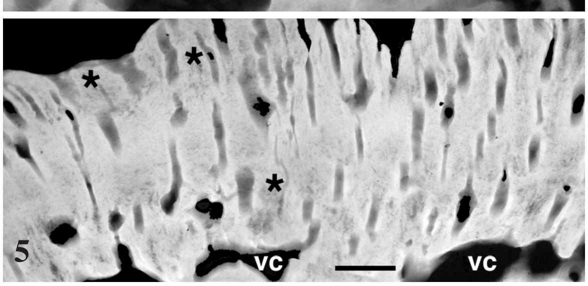
According to the four level of sectioning, it is easy to determine two areas on each section: a) the cortex, with relatively small cavities, and which is the external part of the dentary bone and b) the medulla, with wide cavities, in the middle of the section (see later). The medulla is undiscernible in most cases to the level I (Fig. 3A). From level II to level IV, the medulla diameter progressively grows (Figs 3B-D). Microscopic observation of slides and microradiographs shows that the cortex, as the medulla in the dentary, are constituted by a bony tissue richly vascularised.

At level I, that is located near the mandibular symphysis (joining area of both dentaries), we observe that bone can be partly substituted by cartilage and/or chondroid bone (Fig. 4) whereas away the symphyseal region, cortical bone is mainly fibrillar bone (Fig. 5). In the cortical bone, we distinguish small cavities, which are corresponding to vascular canals. At that level, we observe many pores that are becoming more elongated while reaching the periphery but this sight is perhaps depending on the section plan.

For sections levels II, III and IV, we can easily divide the dentary in two parts (Fig. 3): a central part, the "medulla", including several cavities oftentimes representing wide sizes; a peripheric part, the "cortex", more or less vascularised (Fig. 6). At level IV, the medulla presents large cavities (Fig. 3); among them one, at least, can correspond to the Meckel's cartilage emplacement. At this level the Meckel's cartilage is unmineralized and so wholly transparent to X-rays.

Whatever the localisation, i.e. medular and cortical bone, surface of vascular canals and vascular cavities are either regular, smooth or rough, alveolated. The first ones indicate a recent bony deposition or an active osteogenesis in progress; the second ones are active erosive bays whose





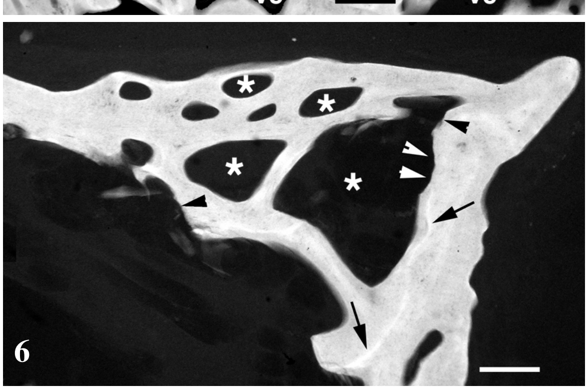


Figure 4. - Section level I. Microradiograph. The section is near the symphysis and it shows cartilage partly mineralised (white arrows) and chondroid bone (black arrows). (b = bone; vc = vascular cavity). (Scale bar = 200 μm). *[Section du niveau I. Microradiographie. La coupe passe à proximité de la symphyse mandibulaire et montre du cartilage partiellement minéralisé (flèches blanches) et de l'os chondroïde (flèches noires). (b = os ; vc = cavité vasculaire). (Échelle = 200 µm).]*

Figure 5. - Section level I. Detail of the cortex in the labial side. Bone is richly vascularised with radial canals and some axial ones. Some areas of bony tissue looks gently less mineralised (black asterisks) because of local numerous osteocytes. (vc = vascular cavities. (Scale bar = 200 μm). *[Section du niveau I. Détail de la face labiale du cortex. Le tissu osseux est richement vascularisé, avec des canaux radiaires et quelques canaux axiaux. Quelques secteurs du tissu osseux sont légèrement moins minéralisés (astérisques noirs) à cause de la présence, localement, de nombreux ostéocytes. (vc = cavité vasculaire). (Échelle = 200 µm).]*

Figure 6. - Section level IV. Detail of the cortical bone showing several vascular canals and cavities (white asterisks), reversal cementing lines (black arrows) and eroded surfaces (arrow heads). (Scale bar = 200 μm). *[Section du niveau IV. Détail de l'os cortical montrant plusieurs canaux et cavités vasculaires (astérisques blancs), des lignes cimentantes de réversion (flèches noires) et des surfaces d'érosion (têtes de flèches). (Échelle = 200 µm).]*

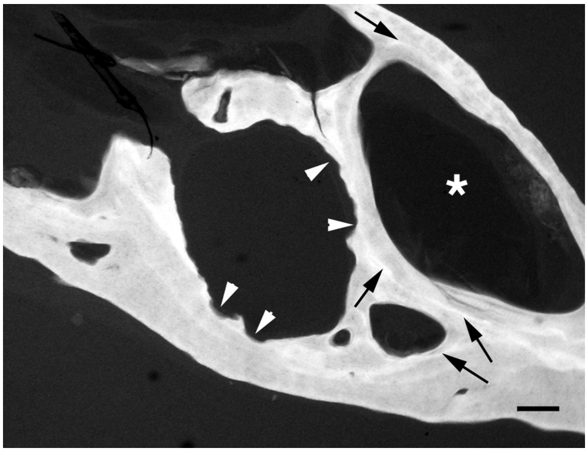


Figure 7. - Section level IV. Detail showing one vascular cavity the walls of which are regularly smooth (asterisk) indicating an osteogenesis phase and an active erosive bay the walls of which are lined by Howship's lacunae (white arrow heads). The black arrows point reversal cementing lines. (Scale bar = 100 µm). *[Section du niveau IV. Détail montrant une cavité vasculaire avec des parois régulières (astérisque) qui indiquent une phase d'ostéogenèse et une baie d'érosion active dont les parois présentent des alvéoles ou lacunes de Howship (têtes de flèches blanches). Les flèches noires pointent des lignes cimentantes de réversion. (Échelle = 100 µm).]*

alveolated patches are true Howship's lacunae that housed clastic cells (Figs 6, 7).

Functional teeth are tightly fused on the dentary by attach bone (Figs 3B, 3C, 8). The bony tissue of the dentary floor located under the teeth shows numerous cementing lines that characterise an important remodelling of bone (Fig. 8). When teeth are lacking the surface of dentary shows more or less irregular trabeculae (Fig. 8, insert). The teeth are regularly replaced. Their base (attach bone and basal dentine) is attacked by clastic cells; the apical part of the teeth falls and the surface of dentary clearly show Howship's lacunae on bony trabeculae (Fig. 8). Then, a new tooth erupts and its base fuses to the dentary bone floor owing to new attach bone.

Dentary mineral rate (DMR)

Dentary relative dry weight (RD1) of each salmon increases significantly during anadromous migration especially with males where the RD1 rises from 2.5 (± 0,18) for the ascending salmons to reach 6.2 (± 1,18) for the spawning salmons (Tab. II). In females the RD1 was 1,9 (± 0,05) for the ascending fish and 2,5 (± 0,17) in spawning ones. The count of the teeth (Fig. 9A) represents a very important data to interpret the results (see discussion below).

The augmentation of the bony weight (RD1) of the dentary goes with an increasing of the mineral weight (RD2) (Tab. II). The quantification of dentary mineral rate (DMR) of the wild salmons showed that for the two sexes there is no significant difference between the ascending salmons and the spawning salmons (Tab. III; Fig. 9A).

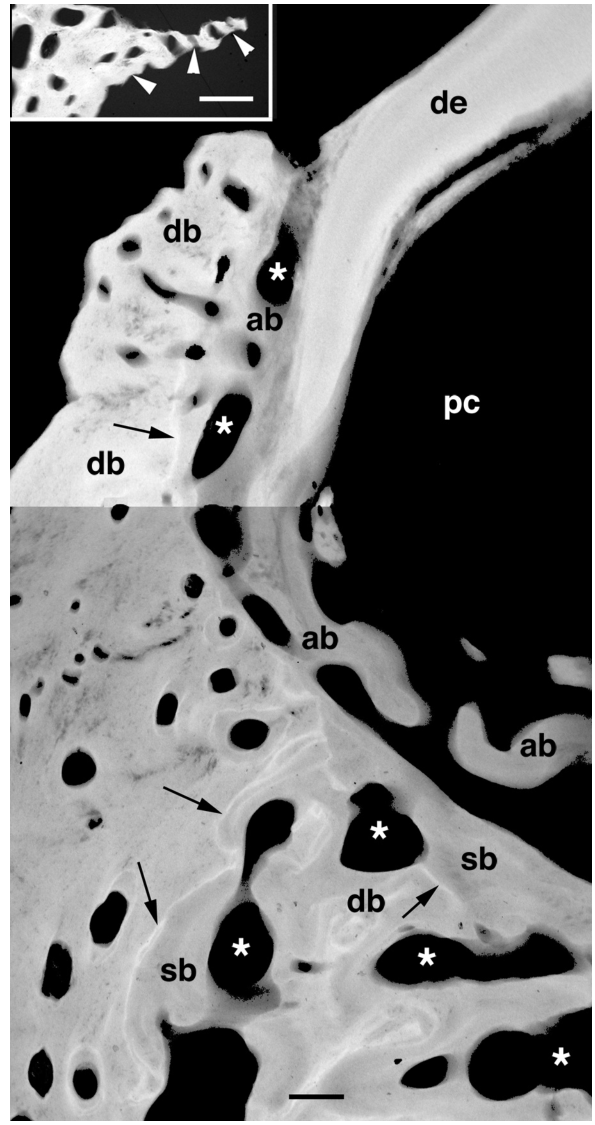


Figure 8. - Section level IV. Detail of the base of a functional teeth and its fixation to the dentary plate owing to attach bone (ab). The bone of the plate shows numerous reversal cementing lines (black arrows) and a lot of vascular cavities (white asterisks) and vascular canals. (db = dentary bone; de = dentine; pc = pulpar cavity; sb = secondary bone). (Scale bar = 150 µm). In the insert, the detail of the attach bone shows Howship's lacunae (arrow heads), after resorption of the base of a tooth. (Scale bar = 150 µm). *[Section du niveau IV. Détail de la base d'une dent fonctionnelle et de l'os d'attache permettant sa fixation sur la plaque dentaire (ab). L'os de la plaque dentaire montre de nombreuses lignes cimentantes de réversion (flèches noires) et un certain nombre de cavités vasculaires (astérisques blancs) et de canaux vasculaires. (db = os dentaire ; de = dentine ; pc = cavité pulpaire ; sb = os secondaire). (Échelle = 150 µm). Dans l'encadré, un détail de l'os d'attache montrant des lacunes de Howship (têtes de flèches), après résorption de la base de la dent fonctionnelle. (Échelle = 150 µm).]*

The same observations were realized with the reared salmons (Tab. IV); the males as the females do not show significant differences (Tab. III). The ratio of the dentary weight

Table II. - Variation of the dentary dry weight (RD1) and the mineral weight (RD2) in wild salmons. *[Variation du poids sec (RD1) et du poids de minéral (RD2) du dentaire chez les saumons sauvages.]*

Migration stage	Ascending salmons ❶		Spawning salmons ❷		Test between ❶ & ❷
	females	Males	Females	Males	
RD1	1.9 ± 0.05	2.5 ± 0.18	2.5 ± 0.17	6.2 ± 1.18	S (p < 0.0001)
RD2	0.77 ± 0.08	0.87 ± 0.16	1.2 ± 0.08	2.89 ± 0.54	S (p < 0.0001)

Table III. - Results of variance analysis (ANOVA) of the dentary mineral rate (DMR) in wild and reared salmons, according to sex, migration stage and age. *[Résultats de l'analyse de variance (ANOVA) du taux de minéralisation du dentaire (DMR) chez les saumons sauvages et chez les saumons d'élevage en fonction du sexe, de l'âge et du stade de migration.]*

		DMR		Significance level
Wild salmons	Variables	F	P	
Sex	Male / Female	F (1.57) = 1.02	0.16	NS
Stage of migration	Ascending / Spawning Salmon	F (1.57) = 0.15	0.73	NS
Age	Grilse / Spring salmon	F (1.57) = 0.25	0.53	NS
Reared salmons	Variables	F	P	
Sex	Male / Female	F (1.31) = 2.09	0.06	NS
Growth stage	Growth /Reproduction	F (1.31) = 21.36	0.0002	S

Table IV. - Variation of the dentary dry weight (RD1) and the mineral weight (RD2) in reared salmons. *[Variation du poids sec (RD1) et du poids de minéral (RD2) du dentaire chez les saumons d'élevage.]*

Growth stage	At the growth stage ❶		After the reproduction❷		Test between ❶ & ❷
	females	males	females	males	
RD1	1.8 ± 0.07	2.5 ± 0.18	3.1 ± 0.11	5,0 ± 0,30	S (p < 0.0001)
RD2	0.93 ± 0.04	1.21 ± 0.07	1.48 ± 0.06	2,33 ± 0,13	S (p < 0.0001)

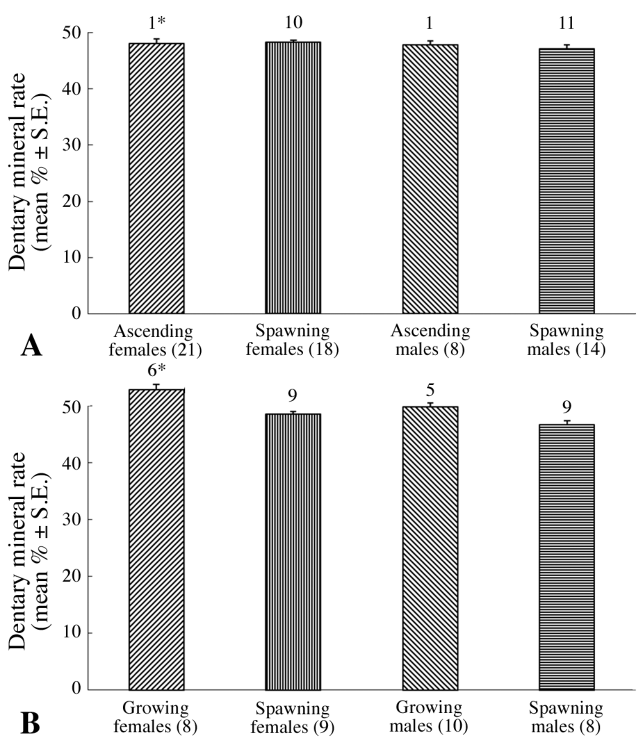


Figure 9. - Dentary mineral rate (DMR) (bar) and number of functional teeth (number on the bar). **A**: Wild salmon; **B**: Reared salmon. *[Taux de minéral du dentaire (histogramme) et nombre de dents fonctionnelles (chiffre au-dessus de l'histogramme).* ***A*** *: Saumons sauvages ;* ***B*** *: Saumons d'élevage.]*

by the fork length (RD1) and the teeth number vary as those of wild salmons: increasing of the dentary weight (RD1) and the teeth number between the growth and the spawning stages (Fig. 9B) in males as in females. However, the latter differences were less important in the case of reared salmons. In return, their dentary mineral rate (DMR) decreases significantly between the growth and the reproduction stage (Tab. III).

Dentary bone compactness (DBC)

Quantitative study of the four sections levels showed that the total compactness of dentaries is minimal for the level IV; it increases progressively to reach its maximum ahead at level I (Fig. 10). The medulla compactness is always low because of its wide cavities; however it rises from level IV to level I from 5% to 20%. For each specimen the cortex compactness is approximately constant for different levels. Therefore, we have used only this cortical compactness as comparison element between salmons sampled at different stages of migration because total compactness fluctuates too considerably with the section level due to the great variability of the medulla compactness.

The comparison of the cortex compactness of the four levels showed that there is no significant difference between levels I, II and III (Fig. 11A). However the compactness average at level IV is less important than the first three lev-

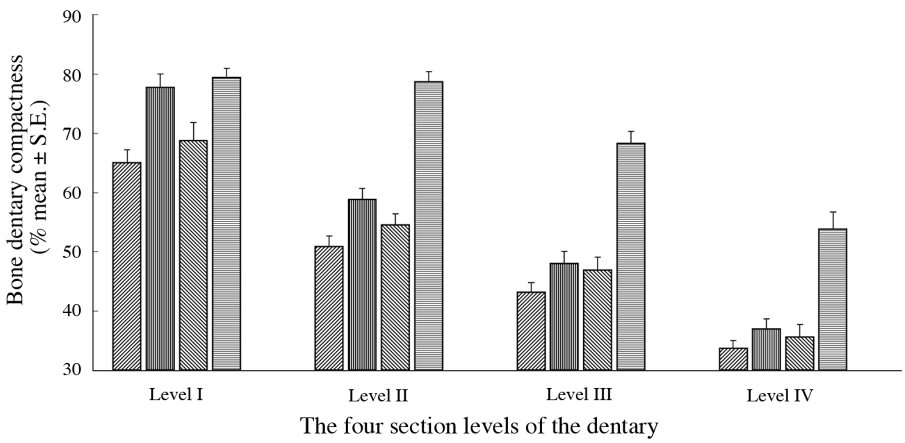


Figure 10. - Bone compactness of the whole dentary (DBC) in ascending and spawning wild salmon according to the sectioning level. (For each level, successively from the left to the right: ascending female, spawning female, ascending male and spawning male). *[Compacité osseuse totale du dentaire (DBC) chez les saumons de montée et en reproduction en fonction du niveau de coupe. (Pour chaque niveau, successivement de la gauche vers la droite : femelle de montée, femelle en reproduction, mâle de montée et mâle en reproduction).]*

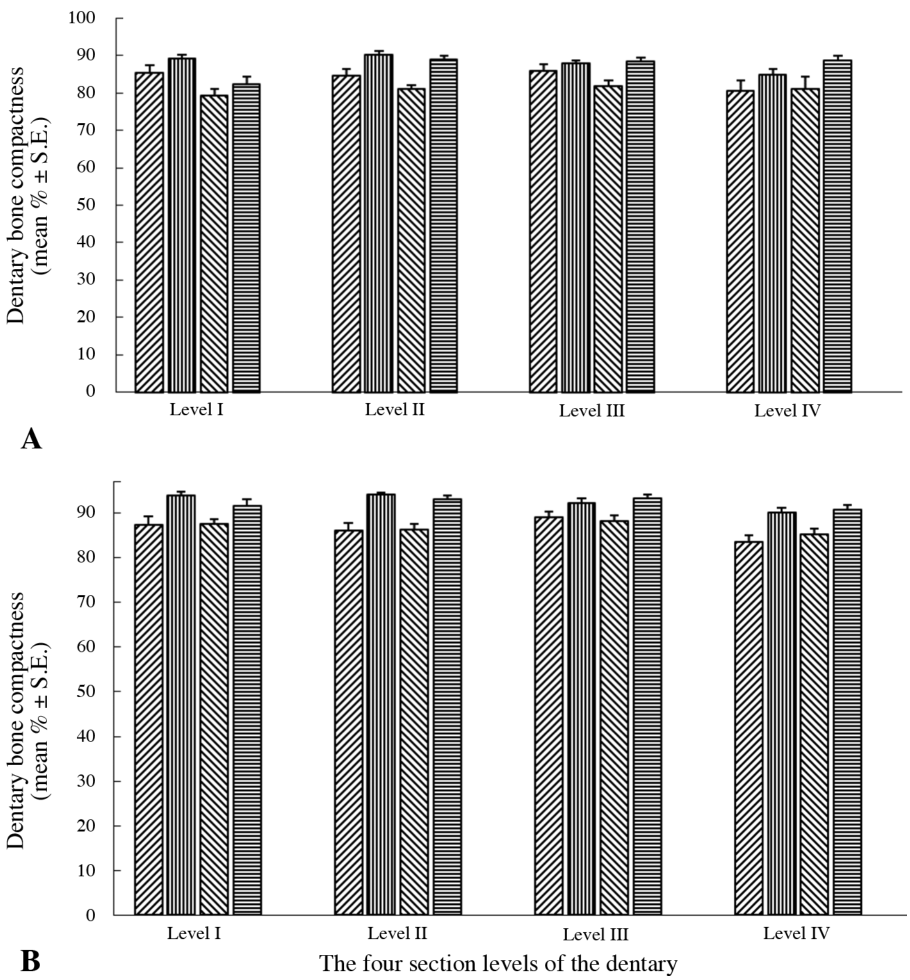


Figure 11. - Cortical bone compactness of the dentary (DBC). **A**: Ascending and spawning wild salmon (for each level, successively from the left to the right: ascending female, spawning female, ascending male and spawning male); and **B**: Reared salmon (for each level, successively from the left to the right: growing female, spawning female, growing male and spawning male). *[Compacité de l'os cortical du dentaire (DBC).* ***A*** *: Saumons sauvages de montée et en reproduction (pour chaque niveau, successivement de la gauche vers la droite: femelle de montée, femelle en reproduction, mâle de montée et mâle en reproduction) ;* ***B*** *: Saumons d'élevage (pour chaque niveau, successivement de la gauche vers la droite: femelle en croissance, femelle en reproduction, mâle en croissance et mâle en reproduction)].*

els. Indeed, the cortex of level IV is constituted by a highly vascular quite thin bone. Variances analyses (Tab. V) have not shown significant differences of cortex compactness between males and females at the same physiological stage for both wild and reared salmons ($p > 0.05\%$). Nevertheless, for the four sections levels analysed, dentary bone compactness is significantly higher for the spawning area salmons than for the ascending ones (difference order about 4% and $p < 0.0001$). These tendencies are also observed with reared salmons: salmons that have reproduced present an increasing compactness (about 6%) higher than that observed with salmons in growth stage (Fig. 11B).

Table V. - Results of variance analysis (ANOVA) of the cortical dentary bone compactness (DBC) in wild and reared salmons, according to sex, migration stage and section level. *[Résultats de l'analyse de variance (ANOVA) de la compacité de l'os cortical du dentaire (DBC) chez les saumons sauvages et d'élevage en fonction du sexe, du stade de migration et du niveau de coupe.]*

Wild salmons	Variables	Cortex compactness		Signification test
		F	P	
Sex	Male / Female	F (1.228) = 6.08	0.19	NS
Migration stage	ascending / spawning area	F (1.228) = 30.36	< 0.0001	S
Section level	N I. N II. N III et N IV	F (3.228) = 1.41	0.24	NS
Reared salmons	Variables	F	P	
Sex	Male / Female	F (1.124) = 0.07	0.25	NS
Growth stage	growth /Reproduction	F (1.124) = 109.75	< 0.0001	S
Section level	N I. N II. N III et N IV	F (3.124) = 6.92	0.09	NS

Table VI. - Results of variance analysis (ANOVA) of the mineralization degree of dentary (MDD) in wild and reared salmons, according to sex, migration stage and age. *[Résultats de l'analyse de variance (ANOVA) du degré de minéralisation (MDD) des dentaires chez les saumons sauvages et d'élevage en fonction du sexe, du stade de migration et de l'âge.]*

Wild salmons	Variables	DDM of dentaries		Signification test
		F	P	
Sex	Male/ Female	F (1.53) = 1.31	0.24	NS
Migration stage	Ascending / spawning area	F (1.53) = 19.77	< 0.0001	S
Age	Castillan / spring salmons	F (1.53) = 0.35	0.13	NS
Reared salmons	Variables	F	P	
Sex	male / female	F (1.31) = 0.19	0.66	NS
Growth stage	growth /reproduction	F (1.31) = 12.16	0.0045	S

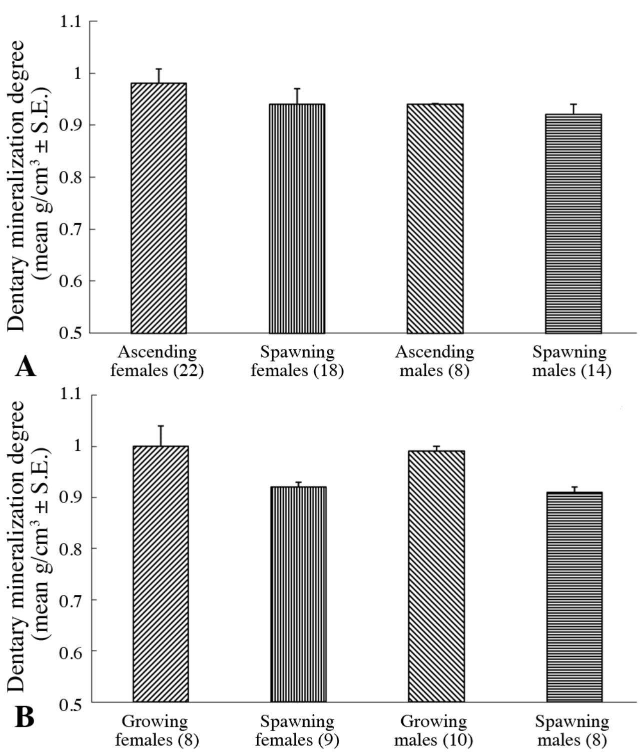


Figure 12. - Mineralization degree of cortical bone (MDD) of the dentary. **A**: Ascending and spawning wild salmon; **B**: Reared salmon. *[Degré de minéralisation de l'os cortical (MDD) du dentaire. **A** : Saumons sauvages de montée et en reproduction ; **B** : Saumons d'élevage.]*

Mineralization degree of dentary (MDD)

The mean mineralization degree fluctuates between 0.92g of hydroxyapatite/cm^3 for the spawning males to 0.98g/cm^3 for the ascending females (Fig. 12). Thanks to the variance analyse (Tab. VI) we have compared the MDD average obtained with dentaries according to sex and migration stage. In the case of wild salmons we notice that the MDD is significantly higher for the ascent salmons than for the spawning salmons. The decrease between the ascending salmons and the spawning ones is about 0.02 and 0.04 g/cm^3. In the female dentaries, MDD is slightly higher than in the males; however statistical tests at a given migration stage have not revealed any significant difference between the two sexes (Tab. VI).

The mineralization degree of dentaries in reared salmons evolves similarly to the wild salmons (from 0.91 g/cm^3 to 1.00 g/cm^3; mean values) with a more important decrease (about 0.08 g of hydroxyapatite/cm^3; mean values) between the growth stage and the post-spawning stage (Fig. 12; Tab. VI). Here also we do not noticed significant difference between males and females.

DISCUSSION

The present study shows two important phenomena:

1. For both males and females, the increasing of the dentary bone compactness level after reproduction, for both

wild and reared salmons, indicates certainly an intense osteogenesis (bone deposit). This bony change is a result of resorption-reconstruction mechanisms. Yet, the latter balance-ratio is positive and could have as origin either an increase of the apposition rate or a decrease of the osteoclastic resorption rate, even both events. Taking into account that the dentary size rises during the maturation stage, notably in males, it seems that the first interpretation is more advisable. This hypothesis could be verified by quantifying separately the active surfaces of osteogenesis and surfaces of resorption.

2. A mineral deficit of the dentary in a whole that seems inconsistent with the development of the kype. This has two explications. First, the construction of the kype is the result of an important osteogenesis and the mineralization of the new bone is probably not complete. But also in the mature bone the quantitative microradiography indicates a slight decrease of the MDD in cortical bone i.e., a slight deficit of mineral. To our opinion, this second phenomenon may be a direct result of a diffuse demineralization process (Kacem and Meunier, 2003). Indeed the osteoclastic action, which releases simultaneously organic elements and mineral ions, generates a null balance-ratio of the dentary mineral rate. Besides, we have remarked that, even if it is present, the processes of osteoclastic resorption were limited in the dentary since the bone compactness is growing. In return, the diffuse demineralization (= halastasy; Lopez, 1973; Kacem and Meunier, 2003) affects just the mineral component keeping unharmed the organic texture, and thus the mineralization rate decreases.

Though, just the males presented the phenomenon of "kype", it is noteworthy to notice that at the tissue level, the dentaries of females presented the same increasing process of bony substance. Tchernavin (1944) have already mentioned a slight increasing tendency of the jaws of female salmons and this may confirm our observations. But in the females, the mineral is removed from vertebrae and scales especially for spawning preparation since calcium has an important function as a vitellogenin solvent (Carragher and Sumpter, 1991; Persson, 1997).

It is important to notice the difference of comportment between the jaw elements such as dentaries, and vertebral axis during migration. The males of salmons, and the females of salmons at second degree, are the subject of transmission of several components belonging to the bony substance from skeletal territories, like vertebrae and scales, to other ones: notably to the dentaries. However, dentaries loss a part of their mineral. From a physiologically point of view, it is obvious that there is a clear specialization of the skeletal effecters. The vertebrae and scales react specifically to the factors included in the control of various phenomenons of sexual maturation during anadromous migration (Persson, 1997; Persson *et al.*, 1998a,b). The comparison of the percentages of the cortex compactness of the dentaries between the spawning salmons and the ascending salmons shows that the balance–ratio of the bony change is positive (increase of the compactness). Such a balance-ratio must be originated by the increasing of the apposition rate (osteogenesis) as we observe a dentary hypertrophy notably in the males during the anadramous migration. This significant increasing of the compactness between the ascending salmons and the spawning salmons is conspicuous for both males and females.

Indeed at the beginning of anadromous migration, the salmons did not present nearly any teeth whereas the spawning area salmons had a complete set of teeth. The development of new teeth implicates an osteogenetic activity at the level of the supporting bone (dentary) and of the attach bone at the base of the tooth (Bergot, 1975; Sire *et al.*, 1990). Thus the establishment of new dentition implies an increasing of the compactness rate of the dentaries in both sexes throughout anadromous migration; this physiological process is more active in reared salmons than in wild salmons (Fig. 11). This probably results from the breeding conditions that were less costly than the migrating activity that needs more metabolic energy

Witten *et al.*, (2005) contested the statement of a complete "teeth loss", followed by a "so-called breeding teeth" stage. They showed that all along this crucial period, salmon has an "every third position" pattern of teeth: a functional tooth goes always with a young functional tooth and a tooth germ. However, they considered that the "complete tooth loss" statement resulted from the maceration techniques. We can agree this assertion but, as a matter of fact, during the ascent of the salmon, it is obvious that all the functional teeth are submitted simultaneously to drastic resorption whereas, normally, this phenomenon does not affect the dentary as a whole. Effectively, the dentition of young salmons (parrs, smolts and post-smolts) studied with the same maceration techniques shows that they all have 9 to 12 functional teeth on each lower jaw (Kacem, 2000). So spawning migration has a true accurate physiological effect that induces a period of time where no, or practically no, functional teeth is tightly attached to the dentary bone: the base of the future new functional teeth are not still definitely fixed by attachment bone to the dentary plate. So during this time, effectively, maceration techniques removed all teeth: the eroded ones, the non-still fixed ones and the tooth germs. We think that the "tooth loss / breeding teeth" process described by Tchernavin (1938b) corresponds to a true specific critic physiological sensibility of the "dentary-teeth" system controlled by spawning of the salmon.

The mean MDD is weakly lower than the mineralization degree of adult vertebrae; the difference varies from 0.04 to 0.08 g/cm^3 (Kacem *et al.*, 2000; Kacem and Meunier, 2003). It is also lower than the MDD of juveniles salmons from 0.3 to 0.4 g/cm^3 (Kacem, 2000). These results show that the

mineral component of bony tissues in salmon varies according the bones and to the physiological states of fishes. The comparison of the MDD of the dentaries has shown that there is significant decrease of the mineral at the histological level, between the ascending and the spawning salmons (Tab. VI; Fig. 12). However, the measurement of the DMR shows that the total dentary mineral does not change throughout anadromous migration (Tab. IV; Fig. 9). This contradictory result is directly linked to the fact that while measuring the DMR of the spawning salmons we have kept the breeding teeth in place; on the spawning fish these teeth are significantly more numerous than on ascending fish (Fig. 9). Yet, these teeth are constituted by a dentine layer, covered by a hypermineralized substance (the enameloid) as the other Salmonidae (Bergot, 1975). Thus, with an equal volume of mineralized tissues, the teeth present more mineral than the bony tissue. Effectively, the incineration of teeth shows that 75% of their dry weight is constituted by mineral, i.e. 20-25 points above mineralization rate of bone. So, this mineral supplement of teeth masks the diffuse demineralization in the incinerated material. Consequently, the MDD reflects in a more precise way the true state of the bone mineralization, since the latter is directly measured at the histological level.

The increasing of the cortical compactness of the dentaries (DBC) is so joined by a reduction of the mineralization rate and degree. This could be explained by a double phenomenon: in the first hand an important stimulation of the osteogenesis in the dentaries throughout sexual maturation, contributing like this to the increasing of bone volume, and in the second hand, the loss of one part of mineral component of the bony substance. The new bone does never reach immediately its maximum mineralization ratio, that it must undergo a maturation. (Ponlot, 1960; Lacroix, 1970; Baud, 1978; Tochon-Danguy and Schönbörner, 1981). This could explain why this bony piece seems to be loosing mineral elements (DMR and MDD) while it shows an important osteogenesis process and while the total mineral quantity of dentaries increases as it was already shown by the RD1 and RD2 ratio. The dentaries of the spawning salmons are as well constituted by a cartilage partly mineralized, and the chondroid bone notably at the mandibular symphysis (Francillon, 1977; Huysseune and Sire, 1990; Meunier and Huysseune, 1992; Witten and Hall, 2002, 2003).

CONCLUSION

The present study confirms that the skeleton of salmon is a complex system the various bony components of which can act differently to the environmental physiological constraints. During the ascending migration, that comes with gonadic maturation, some bones are resorbing (scales, vertebrae) whereas others expands at least partly (anterior part of dentary) owing to active osteogenesis. This means that the osteoblasts, osteocytes and osteoclasts have thorough specific receptors that are able to discriminate their physiological environment and so to control their own cellular activity. In that field, salmon skeleton performs as in other vertebrates, especially the mammals.

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